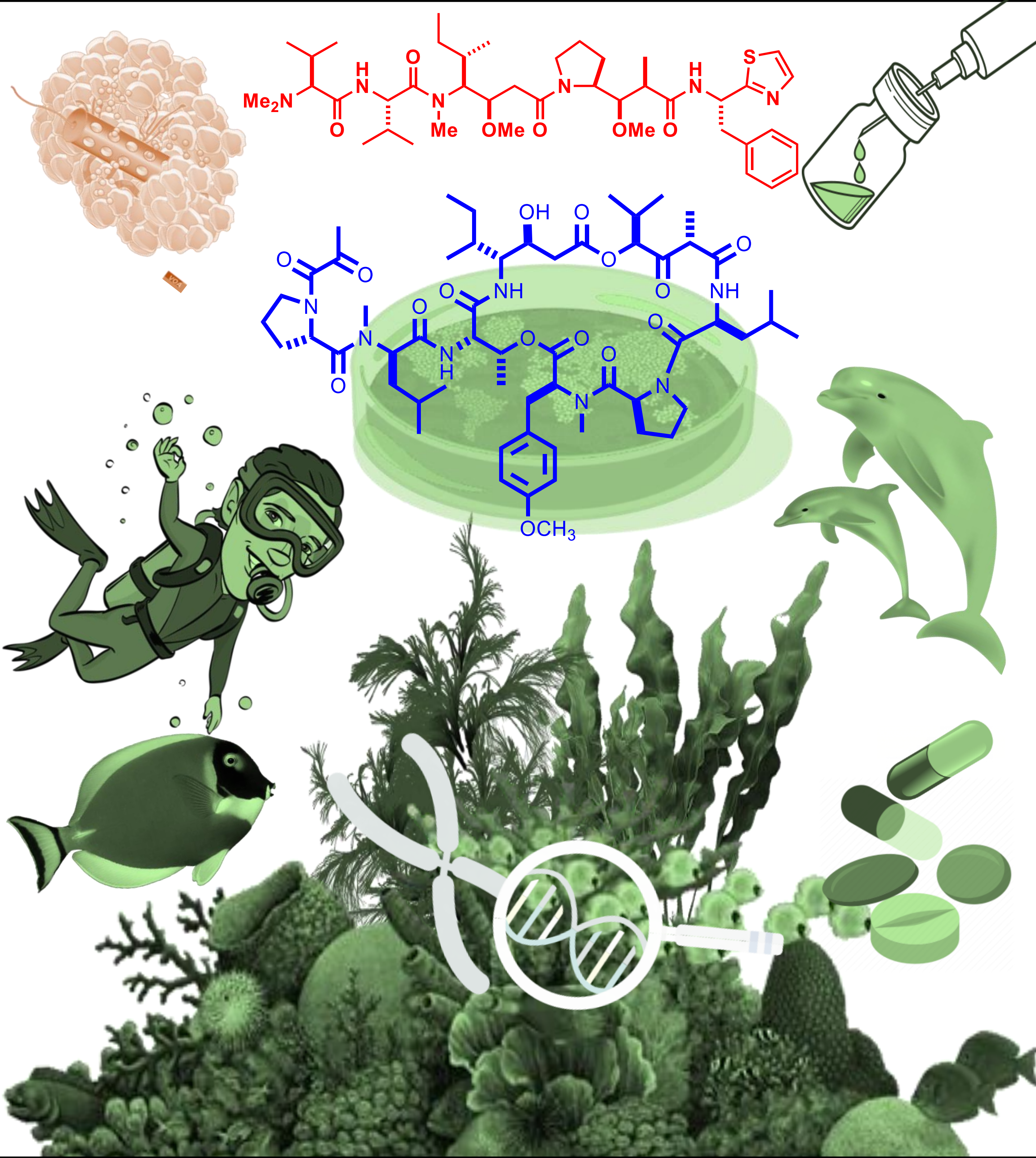
Marine Drugs

2021 – 2022 {Lecture 4}



Dr. Omar M. Sabry

Marine Drugs

III. Marine Pharmaceuticals

Chemical Diversity of Marine Natural Drugs

The nature of compounds from marine organisms are characterized by diverse nature and represent many different structural classes, including linear peptides {**Dolastatin 10**}, cyclic peptides {**ω-Conotoxin}**, cyclic depsipeptides {**Aplidin**}, nucleosides {**Ara C - Ara A**}, polyether or polyketides {**Halichondrin B**}, alkaloids {**Trabectedin**}, PUFAs {**Omega 3 fatty acids**} and terpenoids {**Pseudoterosin A**}.

Peptides: Drugs of future

Peptides are considered as the drugs of future as they have highly specific mode of actions, needed in lower doses, have reduced risk of toxicity, have diverse biological activities e.g. anti-inflammatory activity, anti-HIV activity, antibiotic activity, and anti-cancer activity.

Ribosomal polypeptides {RPPs}and Non Ribosomal polypeptides {NRPPs}

Ribosomal polypeptides: Polypeptides biosynthesized by the routine transcription route {DNA contains gene cluster responsible for biosynthesis of peptides DNA gives mRNA by translation. By translation mRNA gives polypeptides and proteins.

Non-ribosomal polypeptides {NRPP}: No gene cluster responsible for the biosynthesis of Polypeptides was found. It is said that: "**NRPP are biosynthesized by a non-routine route which is constructed by Non ribosomal polypeptide synthases present in the cytoplasm**".

Precautions During Extraction, Isolation, and Use of Polypeptides

- Polypeptides from marine creatures need special precautions during extraction, purification, isolation and use, as they will be damaged {de-naturated} by high PH, low PH and high temperature {higher than 30 ^{0}C}. Extraction of Polypeptides from marine creatures must be carried out as soon as possible from **alcohol preserved organisms** {to inhibit enzyme activity and to prevent hydrolysis of polypeptides}.
- Medical Polypeptides {e.g. Ziconotide} must be preserved in a lyophilized state at low temperature in a refrigerator and constituted directly before use in their solution for injection to avoid hydrolysis.
- Peptides with therapeutic value can't be administered orally nor by I.M injection as Plasma membrane restricts their movement across intestinal epithelial cells and also, they are degraded by protease enzyme.

Depsipeptides

Chain of amino acids {linked by amide groups} alternating with carboxylic acids {linked by ester group}.

Detection of the depsipeptides in normal peptides

Using pepsin enzyme: Depsipeptides will not be completely digested {hydrolyzed}

IR: Carbonyl group in normal peptides can be identified near the range of 1650 cm^{-1}. Carbonyl group in ester group of depsipeptides can be identified near the range of 1730 cm^{-1}

^{13}CNMR: Can't differentiate between Carbonyl group in esters of depsipeptides and amide groups in all polypeptides {range from 165 – 175 ppm for both}

Isolation of Marine Polypeptides

- After extraction with organic solvents e.g. CH_2Cl_2/CH_3OH and upon using VLC {Vacuum Liquid Chromatography} for fractionations of marine organisms extracts, Polypeptides will be eluted mainly in the 80 % Ethyl acetate/hexane fraction and in the 100% ethyl acetate fraction {Mildly polar}.
- During isolation and purification of peptides with HPLC-PDA {Photodiode array detector = DAD} is more preferable than UV detector during separation of due to:

A- Wide range of λ_{max} {200 - 800nm}.

- DAD {PDA} detect nearly all the compounds in the range between 200-800 nm
- UV detector {Single wavelength} can detect peaks at one wavelength only e.g. 280nm UV detector with Variable wavelength detect peaks at multiple wavelengths 3 - 4 wavelengths e.g. 250, 280, 320nm

B- DAD {PDA} gives us an idea about peak purity of the compounds containing conjugated systems while UV detector can't.

Culturing of Marine Microorganisms

- The true source of most of the isolated Marine Natural Products compounds is the symbiotic microorganisms rather than the host. So, it is important for Marine Natural Products scientist to know how to culture marine micro-organisms to isolate the bioactive compound or even the gene cluster from the true producer.
- Upon carrying out microbial culturing of the sea water micro-organisms, only 0.1% of the marine microorganisms will grow {due to differences in the conditions between sea water and nutrient media}.

- HTPS {High Throuput Screening Technique} can be used for growing microorganisms in short time and high numbers.

Identification of Marine Polypeptides

Nin-hydrin: They give blue to purple color with Nin-hydrin

^{1}HNMR:

NH of the amide group appears at 6.40– 6.95 ppm **one proton in integration**

N-CH_3 of the amide group appears at 2.85 – 3.00 ppm **three protons**

Mass Spectroscopy: For determination of molecular weight of the bioactive peptides, ESIMS {**Electro Spray Ionization Mass Spectroscopy**} and FABMS {**Fast Atom Bombardment Mass Spectroscopy**} are more preferable MS techniques in determination of the molecular weight and the sequence of amino acids as they are soft techniques giving gradual fragmentation of the peptides and cyclic depsipeptides. So, we can know the molecular weight and predict the amino acid constituents and their sequence in the polypeptide easily.

EIMS {**Electro Impact Mass Spectroscopy**} is a tough technique giving sudden and complete fragmentation of the cyclic peptides {e.g. complete damage of amino acids}. So we can't predict the amino acids and their sequence.

Detection of aromatic amino acids in depsipeptides

UV spectroscopy: Aromatic amino acids absorb electromagnetic waves in UV range as they have conjugated systems λ_{max} must be more than 280 nm.

IR spectroscopy: C-H aromatic at 3000 - 3200 cm^{-1}

^{1}HNMR: Aromatic protons chemical shift range 6.8 – 8.2 ppm

^{13}CNMR: Aromatic carbons at 110 – 150 ppm

Contribution of the D-amino acids in polypeptides and cyclic depsipeptides makes polypeptides toxic to man {D-amino acids will not be metabolized in human body}.

Determination of the stereochemistry of amino acids in linear and cyclic polypeptides

Can be carried out by hydrolysis of about 20 – 200 µg of the cyclic polypeptides with 6 N HCl in a vial then reaction with {**Marfey's reagent = 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide**}. Hydrolysis Product analyzed using chiral stationary phase HPLC using both standard L- amino acids and D-amino acids reacted with **Marfey's reagent** as references. Then compare retention data of sample amino acids derivatives and standard amino acids derivatives.